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(54) Title: REGULATION OF HUMAN LYSOSTAPHIN-LIKE PROTEASE

(57) Abstract: Reagents which regulate human lysostaphin-like protease activity and reagents which bind to human lysostaphin-like protease gene products can be used to regulate extracellular matrix degradation. Such regulation is particularly useful for treating metastasis of malignant cells, tumor angiogenesis, inflammation, cardiovascular disease, including hypertension, aneurysmal dilatation, and atherosclerosis, neurodegenerative diseases, and pathogenic infections.

REGULATION OF HUMAN LYSOSTAPHIN-LIKE PROTEASE

TECHNICAL FIELD OF THE INVENTION

The invention relates to the area of regulation of extracellular matrix degradation. More particularly, the invention relates to the regulation of human lysostaphin-like protease activity to increase or decrease extracellular matrix degradation.

BACKGROUND OF THE INVENTION

Metastasizing cancer cells invade the extracellular matrix using plasma membrane protrusions that contact and dissolve the matrix with proteases. Agents which inhibit such protease activity can be used to suppress metastases. Proteases also are expressed during development, when degradation of the extracellular matrix is desired. In cases where appropriate extracellular matrix degradation does not occur, supplying a molecule with a protease activity can provide the necessary enzymatic activity. Thus, there is a need in the art for identifying new proteases and methods of regulating extracellular matrix degradation.

SUMMARY OF THE INVENTION

It is an object of the invention to provide reagents and methods of regulating human lysostaphin-like protease activity, for example to regulate degradation of extracellular matrix proteins. These and other objects of the invention are provided by one or more of the embodiments described below.

One embodiment of the invention is a lysostaphin-like protease polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 54% identical to the amino acid sequence shown in SEQ ID NO. 2;

the amino acid sequence shown in SEQ ID NO. 2.

Yet another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a lysostaphin-like protease comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 54% identical to the amino acid sequence shown in SEQ ID NO. 2;

the amino acid sequence shown in SEQ ID NO. 2.1

Binding between the test compound and the lysostaphin-like protease is detected. A test compound which binds to the lysostaphin-like protease is thereby identified as a potential agent for decreasing extracellular matrix degradation. The agent can work by decreasing the activity of the lysostaphin-like protease.

Another embodiment of the invention is a method of screening for agents which decrease extracellular matrix degradation. A test compound is contacted with a polynucleotide encoding a lysostaphin-like protease polypeptide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 54% identical to the nucleotide sequence shown in SEQ ID NO. 1; and

the nucleotide sequence shown in SEQ ID NO. 1.

Binding of the test compound to the polynucleotide is detected. A test compound which binds to the polynucleotide is identified as a potential agent for decreasing extracellular matrix degradation. The agent can work by decreasing the amount of the lysostaphin-like protease through interacting with the lysostaphin-like protease mRNA.

Another embodiment of the invention is a method of screening for agents which regulate extracellular matrix degradation. A test compound is contacted with a lysostaphin-like protease polypeptide comprising an amino acid sequence selected from the group consisting of:

amino acid sequences which are at least about 54% identical to the amino acid sequence shown in SEQ ID NO. 2;

the amino acid sequence shown in SEQ ID NO. 2.

A lysostaphin-like protease activity of the polypeptide is detected. A test compound which increases lysostaphin-like protease activity of the polypeptide relative to lysostaphin-like protease activity in the absence of the test compound is thereby identified as a potential agent for increasing extracellular matrix degradation. A test compound which decreases lysostaphin-like protease activity of the polypeptide relative to lysostaphin-like protease activity in the absence of the test compound is thereby identified as a potential agent for decreasing extracellular matrix degradation.

Even another embodiment of the invention is a method of screening for agents which regulate extracellular matrix degradation. A test compound is contacted with a lysostaphin-like protease product of a polynucleotide which comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 54% identical to the nucleotide sequence shown in SEQ ID NO. 1; and

the nucleotide sequence shown in SEQ ID NO. 1.

Binding of the test compound to the lysostaphin-like protease product is detected. A test compound which binds to the lysostaphin-like protease product is thereby identified as a potential agent for decreasing extracellular matrix degradation.

Still another embodiment of the invention is a method of reducing extracellular matrix degradation. A cell is contacted with a reagent which specifically binds to a polynucleotide encoding a lysostaphin-like protease polypeptide or the product encoded by the polynucleotide, wherein the polynucleotide comprises a nucleotide sequence selected from the group consisting of:

nucleotide sequences which are at least about 54% identical to the nucleotide sequence shown in SEQ ID NO. 1; and

the nucleotide sequence shown in SEQ ID NO. 1.

Lysostaphin-like protease activity in the cell is thereby decreased.

The invention thus provides reagents and methods for regulating human lysostaphin-like protease activity. These methods and reagents can be used, *inter alia*, to suppress metastasis of malignant cells, tumor angiogenesis, inflammation, cardiovascular diseases, including hypertension, aneurysmal dilatation, and atherosclerosis, neurodegenerative diseases, and pathogenic infections.

BRIEF DESCRIPTION OF THE DRAWING

Fig. 1 shows the DNA-sequence encoding a lysostaphin-like protease polypeptide.

Fig. 2 shows the amino acid sequence deduced from the DNA-sequence of SEQ ID NO: 1.

Fig. 3 shows the amino acid sequence of lysostaphin from *Synechocystis sp.*

Fig. 4 shows the alignment of human lysostaphin-like protease (SEQ ID NO. 2) with *Synechocystis sp.* lysostaphin (SEQ ID NO. 3). Underlined region indicates metalloprotease domain.

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to an isolated polynucleotide encoding a heparanase-like enzyme polypeptide and being selected from the group consisting of:

a) a polynucleotide encoding a heparanase-like enzyme polypeptide comprising an amino acid sequence selected from the group consisting of:
amino acid sequences which are at least about 54% identical to the amino acid sequence shown in SEQ ID NO. 2;
the amino acid sequence shown in SEQ ID NO. 2.

b) a polynucleotide comprising the sequence of SEQ ID NO. 1;

c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and (b);

d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code; and

e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d).

Furthermore, it has been discovered by the present applicant that regulators of a lysostaphin-like protease, particularly a human lysostaphin-like protease, can be used to regulate degradation of extracellular matrix proteins. Lysostaphin is a bacterial

zinc metalloprotease that degrades staphylococcal cell wall peptidoglycans and also binds tightly to elastin and contains elastolytic activity. Sequence similarities with matrix metalloproteases (MMP) suggest that the Ala-Ala-Thr-His-Glu sequence in the amino terminus of lysostaphin is involved in elastin degradation. It is now well established that the interaction of tumor cells with elastin is important during invasion and metastasis. Tumor cells have to cross several barriers, including elastin, to migrate from their original site to form secondary tumors. Therefore, tumor invasion and metastasis may be inhibited by using specific inhibitors of human lysostaphin-like protease.

Human lysostaphin-like protease is 53% identical to bacterial lysostaphin having GenBank Accession No. D90904 (SEQ ID NO. 3) and contains a region which corresponds to motifs found in the peptidase family M23/M37 (amino acids 19-90 of SEQ ID NO. 2, underlined in FIG. 1); the presence of this region indicates that human lysostaphin-like protease is a metalloprotease. Human lysostaphin-like protease can be used to degrade extracellular matrix proteins, particularly elastin. This activity can be suppressed, *inter alia*, by molecules which bind to the enzyme, particularly to its active site, or by suppressing expression of the gene encoding the enzyme. Alternatively, if desired a human lysostaphin-like protease function can be supplied to a cell by introducing a human lysostaphin-like protease-encoding polynucleotide into the cell.

Polypeptides

Human lysostaphin-like protease polypeptides according to the invention comprise at least 10, 15, 20, 25, 30, 45, 50, 75, 80, 90, or 100 contiguous amino acids of the amino acid sequence as shown in SEQ ID NO. 2 or a biologically active variant of that sequence, as defined below. A human lysostaphin-like protease polypeptide of the invention therefore can be a portion of a human lysostaphin-like protease molecule, a full-length human lysostaphin-like protease molecule, or a fusion protein comprising all or a portion of a human lysostaphin-like protease molecule.

Most preferably, a human lysostaphin-like protease polypeptide has a metalloprotease activity. Thus, human lysostaphin-like protease polypeptides preferably comprise the metalloprotease domain (amino acids 19-90 of SEQ ID NO. 2) or a biologically active variant of that domain.

Biologically Active Variants

Human lysostaphin-like protease variants which are biologically active, *i.e.*, retain a human lysostaphin-like protease activity, also are human lysostaphin-like protease polypeptides. Preferably, naturally or non-naturally occurring human lysostaphin-like protease variants have amino acid sequences which are at least about 54, preferably about 75, 90, 96, or 98% identical to an amino acid sequence shown in SEQ ID NO. 2. Percent identity between a putative human lysostaphin-like protease variant and an amino acid sequence of SEQ ID NO. 2 is determined using the Blast2 alignment program.

Variations in percent identity can be due, for example, to amino acid substitutions, insertions, or deletions. Amino acid substitutions are defined as one for one amino acid replacements. They are conservative in nature when the substituted amino acid has similar structural and/or chemical properties. Examples of conservative replacements are substitution of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine.

Amino acid insertions or deletions are changes to or within an amino acid sequence. They typically fall in the range of about 1 to 5 amino acids. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological or immunological activity can be found using computer programs well known in the art, such as DNASTAR software. Whether an amino acid change results in a biologically active human lysostaphin-like protease polypeptide can

readily be determined by assaying for human lysostaphin-like protease activity, as described, for example, in Example 2.

Fusion Proteins

Fusion proteins are useful for generating antibodies against human lysostaphin-like protease amino acid sequences and for use in various assay systems. For example, fusion proteins can be used to identify proteins which interact with portions of a human lysostaphin-like protease polypeptide, including its metalloprotease domain (amino acids 19-90 of SEQ ID NO. 2). Methods such as protein affinity chromatography or library-based assays for protein-protein interactions, such as the yeast two-hybrid or phage display systems, can be used for this purpose. Such methods are well known in the art and also can be used as drug screens.

A human lysostaphin-like protease fusion protein comprises two protein segments fused together by means of a peptide bond. The first protein segment comprises at least 10, 15, 20, 25, 30, 45, 50, 75, 80, 90, or 100 contiguous amino acids of SEQ ID NO. 2 or a biologically active variant of that sequence. Preferably, a fusion protein comprises the metalloprotease domain of a human lysostaphin-like protease molecule. Contiguous amino acids for use in a fusion protein can be selected from the amino acid sequence shown in SEQ ID NO. 2 or from a biologically active variant of that sequence, such as those described above. The first protein segment also can comprise full-length human lysostaphin-like protease.

The second protein segment can be a full-length protein or a protein fragment or polypeptide. Proteins commonly used in fusion protein construction include β-galactosidase, β-glucuronidase, green fluorescent protein (GFP), autofluorescent proteins, including blue fluorescent protein (BFP), glutathione-S-transferase (GST), luciferase, horseradish peroxidase (HRP), and chloramphenicol acetyltransferase (CAT). Additionally, epitope tags are used in fusion protein constructions, including histidine (His) tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-

G tags, and thioredoxin (Trx) tags. Other fusion constructions can include maltose binding protein (MBP), S-tag, Lex a DNA binding domain (DBD) fusions, GAL4 DNA binding domain fusions, and herpes simplex virus (HSV) BP16 protein fusions. A fusion protein also can be engineered to contain a cleavage site located between the human lysostaphin-like protease polypeptide-encoding sequence and the heterologous protein sequence, so that the human lysostaphin-like protease polypeptide can be cleaved and purified away from the heterologous moiety.

A fusion protein can be synthesized chemically, as is known in the art. Preferably, a fusion protein is produced by covalently linking two protein segments or by standard procedures in the art of molecular biology. Recombinant DNA methods can be used to prepare fusion proteins, for example, by making a DNA construct which comprises coding sequences selected from SEQ ID NO. 1 in proper reading frame with nucleotides encoding the second protein segment and expressing the DNA construct in a host cell, as is known in the art. Many kits for constructing fusion proteins are available from companies such as Promega Corporation (Madison, WI), Stratagene (La Jolla, CA), CLONTECH (Mountain View, CA), Santa Cruz Biotechnology (Santa Cruz, CA), MBL International Corporation (MIC; Watertown, MA), and Quantum Biotechnologies (Montreal, Canada; 1-888-DNA-KITS).

Identification of Species Homologs

Species homologs of human lysostaphin-like protease can be obtained using human lysostaphin-like protease polynucleotides (described below) to make suitable probes or primers to screening cDNA expression libraries from other species, such as mice, monkeys, or yeast, identifying cDNAs which encode homologs of human lysostaphin-like protease, and expressing the cDNAs as is known in the art.

Polynucleotides

A human lysostaphin-like protease polynucleotide can be single- or double-stranded and comprises a coding sequence or the complement of a coding sequence for a human lysostaphin-like protease polypeptide. A partial coding sequence of a human lysostaphin-like protease polynucleotide is shown in SEQ ID NO. 1.

Degenerate nucleotide sequences encoding human lysostaphin-like protease polypeptides, as well as homologous nucleotide sequences which are at least about 50, preferably about 75, 90, 96, or 98% identical to the nucleotide sequence shown in SEQ ID NO. 1 also are human lysostaphin-like protease polynucleotides. Percent sequence identity between the sequences of two polynucleotides is determined using computer programs such as ALIGN which employ the FASTA algorithm, using an affine gap search with a gap open penalty of -12 and a gap extension penalty of -2. Complementary DNA (cDNA) molecules, species homologs, and variants of human lysostaphin-like protease polynucleotides which encode biologically active human lysostaphin-like protease polypeptides also are human lysostaphin-like protease polynucleotides.

Identification of Variants and Homologs

Variants and homologs of the human lysostaphin-like protease polynucleotides disclosed above also are human lysostaphin-like protease polynucleotides. Typically, homologous human lysostaphin-like protease polynucleotide sequences can be identified by hybridization of candidate polynucleotides to known human lysostaphin-like protease polynucleotides under stringent conditions, as is known in the art. For example, using the following wash conditions--2X SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0), 0.1% SDS, room temperature twice, 30 minutes each; then 2X SSC, 0.1% SDS, 50°C once, 30 minutes; then 2X SSC, room temperature twice, 10 minutes each--homologous sequences can be identified which contain at most about 25-30% basepair mismatches. More preferably, homologous

nucleic acid strands contain 15-25% basepair mismatches, even more preferably 5-15% basepair mismatches.

Species homologs of the human lysostaphin-like protease polynucleotides disclosed herein can be identified by making suitable probes or primers and screening cDNA expression libraries from other species, such as mice, monkeys, or yeast. Human variants of human lysostaphin-like protease polynucleotides can be identified, for example, by screening human cDNA expression libraries. It is well known that the T_m of a double- stranded DNA decreases by 1-1.5°C with every 1% decrease in homology (Bonner *et al.*, *J. Mol. Biol. 81*, 123 (1973). Variants of human lysostaphin-like protease polynucleotides or human lysostaphin-like protease polynucleotides of other species can therefore be identified by hybridizing a putative homologous human lysostaphin-like protease polynucleotide with a polynucleotide having a nucleotide sequence of SEQ ID NO. 1 to form a test hybrid. The melting temperature of the test hybrid is compared with the melting temperature of a hybrid comprising human lysostaphin-like protease polynucleotides having perfectly complementary nucleotide sequences, and the number or percent of basepair mismatches within the test hybrid is calculated.

Nucleotide sequences which hybridize to human lysostaphin-like protease polynucleotides or their complements following stringent hybridization and/or wash conditions also are human lysostaphin-like protease polynucleotides. Stringent wash conditions are well known and understood in the art and are disclosed, for example, in Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, 2d ed., 1989, at pages 9.50-9.51.

Typically, for stringent hybridization conditions a combination of temperature and salt concentration should be chosen that is approximately 12-20°C below the calculated T_m of the hybrid under study. The T_m of a hybrid between a human lysostaphin-like protease polynucleotide having a nucleotide sequence shown in SEQ ID NO. 1 and a polynucleotide sequence which is at least about 50, preferably about

75, 90, 96, or 98% identical to that nucleotide sequence can be calculated, for example, using the equation of Bolton and McCarthy, *Proc. Natl. Acad. Sci. U.S.A. 48*, 1390 (1962):

$T_m = 81.5°C - 16.6(\log_{10}[Na^+]) + 0.41(\%G + C) - 0.63(\%formamide) - 600/l)$,

where l = the length of the hybrid in basepairs.

Stringent wash conditions include, for example, 4X SSC at 65°C, or 54% formamide, 4X SSC at 42°C, or 0.5X SSC, 0.1% SDS at 65°C. Highly stringent wash conditions include, for example, 0.2X SSC at 65°C.

Preparation of Polynucleotides

A naturally occurring human lysostaphin-like protease polynucleotide can be isolated free of other cellular components such as membrane components, proteins, and lipids. Polynucleotides can be made by a cell and isolated using standard nucleic acid purification techniques, synthesized using an amplification technique, such as the polymerase chain reaction (PCR), or synthesized using an automatic synthesizer. Methods for isolating polynucleotides are routine and are known in the art. Any such technique for obtaining a polynucleotide can be used to obtain isolated human lysostaphin-like protease polynucleotides. For example, restriction enzymes and probes can be used to isolate polynucleotide fragments which comprise human lysostaphin-like protease nucleotide sequences. Isolated polynucleotides are in preparations which are free or at least 70, 80, or 90% free of other molecules.

Human lysostaphin-like protease cDNA molecules can be made with standard molecular biology techniques, using human lysostaphin-like protease mRNA as a template. Human lysostaphin-like protease cDNA molecules can thereafter be replicated using molecular biology techniques known in the art and disclosed in manuals such as Sambrook *et al.* (1989). An amplification technique, such as PCR,

can be used to obtain additional copies of human lysostaphin-like protease polynucleotides, using either human genomic DNA or cDNA as a template.

Alternatively, synthetic chemistry techniques can be used to synthesize human lysostaphin-like protease polynucleotides. The degeneracy of the genetic code allows alternate nucleotide sequences to be synthesized which will encode a human lysostaphin-like protease polypeptide having, for example, the amino acid sequence shown in SEQ ID NO. 2 or a biologically active variant of that sequence.

Obtaining Full-Length Polynucleotides

The partial sequence of SEQ ID NO. 1 or its complement can be used to identify the corresponding full length gene from which they were derived. The partial sequences can be nick-translated or end-labeled with ^{32}P using polynucleotide kinase using labeling methods known to those with skill in the art (BASIC METHODS IN MOLECULAR BIOLOGY, Davis *et al.*, eds., Elsevier Press, N.Y., 1986). A lambda library prepared from human tissue can be directly screened with the labeled sequences of interest or the library can be converted en masse to pBluescript (Stratagene Cloning Systems, La Jolla, Calif. 92037) to facilitate bacterial colony screening (see Sambrook *et al.*, 1989, pg. 1.20).

Both methods are well known in the art. Briefly, filters with bacterial colonies containing the library in pBluescript or bacterial lawns containing lambda plaques are denatured, and the DNA is fixed to the filters. The filters are hybridized with the labeled probe using hybridization conditions described by Davis *et al.*, 1986. The partial sequences, cloned into lambda or pBluescript, can be used as positive controls to assess background binding and to adjust the hybridization and washing stringencies necessary for accurate clone identification. The resulting autoradiograms are compared to duplicate plates of colonies or plaques; each exposed spot corresponds to a positive colony or plaque. The colonies or plaques are selected and

expanded, and the DNA is isolated from the colonies for further analysis and sequencing.

Positive cDNA clones are analyzed to determine the amount of additional sequence they contain using PCR with one primer from the partial sequence and the other primer from the vector. Clones with a larger vector-insert PCR product than the original partial sequence are analyzed by restriction digestion and DNA sequencing to determine whether they contain an insert of the same size or similar as the mRNA size determined from Northern blot Analysis.

Once one or more overlapping cDNA clones are identified, the complete sequence of the clones can be determined, for example after exonuclease III digestion (McCombie *et al.*, *Methods 3*, 33-40, 1991). A series of deletion clones are generated, each of which is sequenced. The resulting overlapping sequences are assembled into a single contiguous sequence of high redundancy (usually three to five overlapping sequences at each nucleotide position), resulting in a highly accurate final sequence.

Various PCR-based methods can be used to extend the nucleic acid sequences encoding the disclosed portions of human lysostaphin-like protease to detect upstream sequences such as promoters and regulatory elements. For example, restriction-site PCR uses universal primers to retrieve unknown sequence adjacent to a known locus (Sarkar, *PCR Methods Applic. 2*, 318-322, 1993). Genomic DNA is first amplified in the presence of a primer to a linker sequence and a primer specific to the known region. The amplified sequences are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR also can be used to amplify or extend sequences using divergent primers based on a known region (Triglia *et al.*, *Nucleic Acids Res. 16*, 8186, 1988). Primers

can be designed using commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, Minn.), to be 22-30 nucleotides in length, to have a GC content of 54% or more, and to anneal to the target sequence at temperatures about 68-72 ° C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Another method which can be used is capture PCR, which involves PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome DNA (Lagerstrom *et al.*, *PCR Methods Applic. 1*, 111-119, 1991). In this method, multiple restriction enzyme digestions and ligations are used to place an engineered double-stranded sequence into an unknown fragment of the DNA molecule before performing PCR.

Another method which can be used to retrieve unknown sequences is that of Parker *et al.*, *Nucleic Acids Res. 19*, 3055-3060, 1991. Additionally, PCR, nested primers, and PROMOTERFINDER libraries (CLONTECH, Palo Alto, Calif.) can be used to walk genomic DNA. This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Also, random-primed libraries are preferable, in that they will contain more sequences which contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries can be useful for extension of sequence into 5' non-transcribed regulatory regions.

Commercially available capillary electrophoresis systems can be used to analyze the size or confirm the nucleotide sequence of PCR or sequencing products. For example, capillary sequencing can employ flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser

activated, and detection of the emitted wavelengths by a charge coupled device camera. Output/light intensity can be converted to electrical signal using appropriate software (*e.g.* GENOTYPER and Sequence NAVIGATOR, Perkin Elmer), and the entire process from loading of samples to computer analysis and electronic data display can be computer controlled. Capillary electrophoresis is especially preferable for the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample.

Obtaining Polypeptides

Human lysostaphin-like protease polypeptides can be obtained, for example, by purification from human cells, by expression of human lysostaphin-like protease polynucleotides, or by direct chemical synthesis.

Protein Purification

Human lysostaphin-like protease polypeptides can be purified from human cells, such as primary tumor cells, metastatic cells, or cancer cell lines (*e.g.*, colon cancer cell lines HCT116, DLD1, HT29, Caco2, SW837, SW480, and RKO, breast cancer cell lines 21-PT, 21-MT, MDA-468, SK-BR3, and BT-474, the A549 lung cancer cell line, or the H392 glioblastoma cell line). Anaplastic oligodendroglioma is a particularly useful source of human lysostaphin-like protease polypeptides. A purified human lysostaphin-like protease polypeptide is separated from other compounds which normally associate with the human lysostaphin-like protease polypeptide in the cell, such as certain proteins, carbohydrates, or lipids, using methods well-known in the art. Such methods include, but are not limited to, size exclusion chromatography, ammonium sulfate fractionation, ion exchange chromatography, affinity chromatography, and preparative gel electrophoresis. A preparation of purified human lysostaphin-like protease polypeptides is at least 80% pure; preferably, the preparations are 90%, 95%, or 99% pure. Purity of the preparations can be assessed by any means known in the art, such as SDS-

polyacrylamide gel electrophoresis. Enzymatic activity of the purified preparations can be assayed, for example, as described in Example 2.

Expression of Polynucleotides

To express a human lysostaphin-like protease polynucleotide, the polynucleotide can be inserted into an expression vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art can be used to construct expression vectors containing sequences encoding human lysostaphin-like protease polypeptides and appropriate transcriptional and translational control elements. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described, for example, in Sambrook *et al.* (1989) and Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, N.Y, 1989.

A variety of expression vector/host systems can be utilized to contain and express sequences encoding a human lysostaphin-like protease polypeptide. These include, but are not limited to, microorganisms, such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors, insect cell systems infected with virus expression vectors (*e.g.*, baculovirus), plant cell systems transformed with virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (*e.g.*, Ti or pBR322 plasmids), or animal cell systems.

The control elements or regulatory sequences are those non-translated regions of the vector -- enhancers, promoters, 5' and 3' untranslated regions -- which interact with host cellular proteins to carry out transcription and translation. Such elements can vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including

constitutive and inducible promoters, can be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the BLUESCRIPT phagemid (Stratagene, LaJolla, Calif.) or pSPORT1 plasmid (Life Technologies) and the like can be used. The baculovirus polyhedrin promoter can be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (*e.g.*, heat shock, RUBISCO, and storage protein genes) or from plant viruses (*e.g.*, viral promoters or leader sequences) can be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of a nucleotide sequence encoding a human lysostaphin-like protease polypeptide, vectors based on SV40 or EBV can be used with an appropriate selectable marker.

Bacterial and Yeast Expression Systems

In bacterial systems, a number of expression vectors can be selected depending upon the use intended for the human lysostaphin-like protease polypeptide. For example, when a large quantity of a human lysostaphin-like protease polypeptide is needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified can be used. Such vectors include, but are not limited to, multifunctional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene), in which the sequence encoding the human lysostaphin-like protease polypeptide can be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of β-galactosidase so that a hybrid protein is produced. pIN vectors (Van Heeke & Schuster, *J. Biol. Chem. 264*, 5503-5509, 1989 or pGEX vectors (Promega, Madison, Wis.) can be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems can be designed to include heparin,

thrombin, or factor Xa protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH can be used. For reviews, see Ausubel *et al.* (1989) and Grant *et al.*, *Methods Enzymol. 153*, 516-544, 1987.

Plant and Insect Expression Systems

If plant expression vectors are used, the expression of sequences encoding human lysostaphin-like protease polypeptides can be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV can be used alone or in combination with the omega leader sequence from TMV (Takamatsu *EMBO J. 6*, 307-311, 1987). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters can be used (Coruzzi *et al.*, *EMBO J. 3*, 1671-1680, 1984; Broglie *et al.*, *Science 224*, 838-843, 1984; Winter *et al.*, *Results Probl. Cell Differ. 17*, 85-105, 1991). These constructs can be introduced into plant cells by direct DNA transformation or by pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (see, for example, Hobbs or Murray, in MCGRAW HILL YEARBOOK OF SCIENCE AND TECHNOLOGY, McGraw Hill, New York, N.Y., pp. 191-196, 1992).

An insect system also can be used to express a human lysostaphin-like protease polypeptide. For example, in one such system *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. Sequences encoding human lysostaphin-like protease polypeptides can be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of human lysostaphin-like protease polypeptides will render the polyhedrin gene inactive and produce recombinant virus lacking coat

protein. The recombinant viruses can then be used to infect, for example, *S. frugiperda cells* or *Trichoplusia* larvae in which human lysostaphin-like protease polypeptides can be expressed (Engelhard *et al.*, *Proc. Nat. Acad. Sci. 91*, 3224-27, 1994).

Mammalian Expression Systems

A number of viral-based expression systems can be utilized in mammalian host cells. For example, if an adenovirus is used as an expression vector, sequences encoding human lysostaphin-like protease polypeptides can be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome can be used to obtain a viable virus which is capable of expressing a human lysostaphin-like protease polypeptide in infected host cells (Logan & Shenk, *Proc. Natl. Acad. Sci. 81*, 3655-3659, 1984). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, can be used to increase expression in mammalian host cells.

Human artificial chromosomes (HACs) also can be used to deliver larger fragments of DNA than can be contained and expressed in a plasmid. HACs of 6M to 10M are constructed and delivered to cells via conventional delivery methods (*e.g.*, liposomes, polycationic amino polymers, or vesicles).

Specific initiation signals also can be used to achieve more efficient translation of sequences encoding human lysostaphin-like protease polypeptides. Such signals include the ATG initiation codon and adjacent sequences.. In cases where sequences encoding a human lysostaphin-like protease polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals (including the ATG initiation codon) should be provided.

The initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used (see Scharf *et al.*, *Results Probl. Cell Differ. 20*, 125-162, 1994).

Host Cells

A host cell strain can be chosen for its ability to modulate the expression of the inserted sequences or to process an expressed human lysostaphin-like protease polypeptide in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the polypeptide also can be used to facilitate correct insertion, folding and/or function. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (*e.g.*, CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC; 10801 University Boulevard, Manassas, VA 20110-2209) and can be chosen to ensure the correct modification and processing of the foreign protein.

Stable expression is preferred for long-term, high-yield production of recombinant proteins. For example, cell lines which stably express human lysostaphin-like protease polypeptides can be transformed using expression vectors which can contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells can be allowed to grow for 1-2 days in an enriched medium before they are switched to a selective medium. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced human lysostaphin-

like protease sequences. Resistant clones of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems can be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler *et al.*, *Cell 11*, 223-32, 1977) and adenine phosphoribosyltransferase (Lowy *et al.*, *Cell 22*, 817-23, 1980). Genes which can be employed in *tk*$^-$ or *aprt*$^-$ cells, respectively. Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate (Wigler *et al.*, *Proc. Natl. Acad. Sci. 77*, 3567-70, 1980); *npt* confers resistance to the aminoglycosides, neomycin and G-418 (Colbere-Garapin *et al.*, *J. Mol. Biol. 150*, 1-14, 1981); *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murray, 1992 *supra*). Additional selectable genes have been described, for example *trpB*, which allows cells to utilize indole in place of tryptophan, or *hisD*, which allows cells to utilize histinol in place of histidine (Hartman & Mulligan, *Proc. Natl. Acad. Sci. 85*, 8047-51, 1988). Visible markers such as anthocyanins, β-glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, can be used to identify transformants and to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes *et al.*, *Methods Mol. Biol. 55*, 121-31, 1995).

Detecting Expression of Polypeptides

Although the presence of marker gene expression suggests that the human lysostaphin-like protease polynucleotide is also present, its presence and expression may need to be confirmed. For example, if a sequence encoding a human lysostaphin-like protease polypeptide is inserted within a marker gene sequence, transformed cells containing sequences which encode a human lysostaphin-like protease polypeptide can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding a human lysostaphin-like protease polypeptide under the control of a single promoter.

Expression of the marker gene in response to induction or selection usually indicates expression of the human lysostaphin-like protease polynucleotide.

Alternatively, host cells which contain a human lysostaphin-like protease polynucleotide and which express a human lysostaphin-like protease polypeptide can be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassay or immunoassay techniques which include membrane, solution, or chip-based technologies for the detection and/or quantification of nucleic acid or protein.

The presence of a polynucleotide sequence encoding a human lysostaphin-like protease polypeptide can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes or fragments or fragments of polynucleotides encoding a human lysostaphin-like protease polypeptide. Nucleic acid amplification-based assays involve the use of oligonucleotides selected from sequences encoding a human lysostaphin-like protease polypeptide to detect transformants which contain a human lysostaphin-like protease polynucleotide.

A variety of protocols for detecting and measuring the expression of a human lysostaphin-like protease polypeptide, using either polyclonal or monoclonal antibodies specific for the polypeptide, are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay using monoclonal antibodies reactive to two non-interfering epitopes on a human lysostaphin-like protease polypeptide can be used, or a competitive binding assay can be employed. These and other assays are described in Hampton *et al.*, SEROLOGICAL METHODS: A LABORATORY MANUAL, APS Press, St. Paul, Minn., 1990) and Maddox *et al.*, *J. Exp. Med. 158*, 1211-16, 1983).

A wide variety of labels and conjugation techniques are known by those skilled in the art and can be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding human lysostaphin-like protease polypeptides include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, sequences encoding a human lysostaphin-like protease polypeptide can be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and can be used to synthesize RNA probes *in vitro* by addition of labeled nucleotides and an appropriate RNA polymerase, such as T7, T3, or SP6. These procedures can be conducted using a variety of commercially available kits (Amersham Pharmacia Biotech, Promega, and US Biochemical). Suitable reporter molecules or labels which can be used for ease of detection include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Expression and Purification of Polypeptides

Host cells transformed with nucleotide sequences encoding a human lysostaphin-like protease polypeptide can be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The polypeptide produced by a transformed cell can be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode human lysostaphin-like protease polypeptides can be designed to contain signal sequences which direct secretion of human lysostaphin-like protease polypeptides through a prokaryotic or eukaryotic cell membrane.

Other constructions can be used to join a sequence encoding a human lysostaphin-like protease polypeptide to a nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating

domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). The inclusion of cleavable linker sequences such as those specific for Factor Xa or enterokinase (Invitrogen, San Diego, CA) between the purification domain and the human lysostaphin-like protease polypeptide can be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing a human lysostaphin-like protease polypeptide and 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on IMAC (immobilized metal ion affinity chromatography as described in Porath *et al.*, *Prot. Exp. Purif. 3*, 263-281, 1992), while the enterokinase cleavage site provides a means for purifying the human lysostaphin-like protease polypeptide from the fusion protein. Vectors which contain fusion proteins are disclosed in Kroll *et al.*, *DNA Cell Biol. 12*, 441-453, 1993).

Chemical Synthesis

Sequences encoding a human lysostaphin-like protease polypeptide can be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers *et al.*, *Nucl. Acids Res. Symp. Ser.* 215-223, 1980; Horn *et al. Nucl. Acids Res. Symp. Ser.* 225-232, 1980). Alternatively, a human lysostaphin-like protease polypeptide itself can be produced using chemical methods to synthesize its amino acid sequence. Human lysostaphin-like protease polypeptides can be produced by direct peptide synthesis using solid-phase techniques (Merrifield, *J. Am. Chem. Soc. 85*, 2149-2154, 1963; Roberge *et al.*, *Science 269*, 202-204, 1995). Protein synthesis can be performed using manual techniques or by automation. Automated synthesis can be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Various fragments of human lysostaphin-like protease polypeptides can be synthesized separately using chemical methods to produce a full-length molecule.

The newly synthesized peptide can be substantially purified by preparative high performance liquid chromatography (*e.g.*, Creighton, PROTEINS: STRUCTURES AND MOLECULAR PRINCIPLES, WH Freeman and Co., New York, N.Y., 1983). The composition of a synthetic human lysostaphin-like protease polypeptide can be confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure; *see* Creighton, *supra*). Additionally, any portion of the amino acid sequence of the human lysostaphin-like protease polypeptide can be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins to produce a variant polypeptide or a fusion protein.

Production of Altered Polypeptides

As will be understood by those of skill in the art, it may be advantageous to produce human lysostaphin-like protease polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce an RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

The nucleotide sequences disclosed herein can be engineered using methods generally known in the art to alter human lysostaphin-like protease polypeptide-encoding sequences for a variety of reasons, including modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides can be used to engineer the nucleotide sequences. For example, site-directed mutagenesis can be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, introduce mutations, and so forth.

Antibodies

Any type of antibody known in the art can be generated to bind specifically to an epitope of a human lysostaphin-like protease polypeptide. "Antibody" as used herein includes intact immunoglobulin molecules, as well as fragments thereof, such as Fab, $F(ab')_2$, and Fv, which are capable of binding an epitope of a human lysostaphin-like protease polypeptide. Typically, at least 6, 8, 10, or 12 contiguous amino acids are required to form an epitope. However, epitopes which involve non-contiguous amino acids may require more, *e.g.*, at least 15, 25, or 50 amino acids.

An antibody which specifically binds to an epitope of a human lysostaphin-like protease polypeptide can be used therapeutically, as well as in immunochemical assays, including but not limited to Western blots, ELISAs, radioimmunoassays, immunohistochemical assays, immunoprecipitations, or other immunochemical assays known in the art. Various immunoassays can be used to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays are well known in the art. Such immunoassays typically involve the measurement of complex formation between an immunogen and an antibody which specifically binds to the immunogen.

Typically, an antibody which specifically binds to a human lysostaphin-like protease polypeptide provides a detection signal at least 5-, 10-, or 20-fold higher than a detection signal provided with other proteins when used in an immunochemical assay. Preferably, antibodies which specifically bind to human lysostaphin-like protease polypeptides do not detect other proteins in immunochemical assays and can immunoprecipitate a human lysostaphin-like protease polypeptide from solution.

Human lysostaphin-like protease polypeptides can be used to immunize a mammal, such as a mouse, rat, rabbit, guinea pig, monkey, or human, to produce polyclonal antibodies. If desired, a human lysostaphin-like protease polypeptide can be conjugated to a carrier protein, such as bovine serum albumin, thyroglobulin, and

keyhole limpet hemocyanin. Depending on the host species, various adjuvants can be used to increase the immunological response. Such adjuvants include, but are not limited to, Freund's adjuvant, mineral gels (*e.g.*, aluminum hydroxide), and surface active substances (*e.g.* lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol). Among adjuvants used in humans, BCG (*bacilli Calmette-Guerin*) and *Corynebacterium parvum* are especially useful.

Monoclonal antibodies which specifically bind to a human lysostaphin-like protease polypeptide can be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These techniques include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (Kohler *et al.*, *Nature 256*, 495-497, 1985; Kozbor *et al.*, *J. Immunol. Methods 81*, 31-42, 1985; Cote *et al.*, *Proc. Natl. Acad. Sci. 80*, 2026-2030, 1983; Cole *et al.*, *Mol. Cell Biol. 62*, 109-120, 1984).

In addition, techniques developed for the production of "chimeric antibodies," the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used (Morrison *et al.*, *Proc. Natl. Acad. Sci. 81*, 6851-55, 1984; Neuberger *et al.*, *Nature 312*, 604-08, 1984; Takeda *et al.*, *Nature 314*, 452-54, 1985). Monoclonal and other antibodies also can be "humanized" to prevent a patient from mounting an immune response against the antibody when it is used therapeutically. Such antibodies may be sufficiently similar in sequence to human antibodies to be used directly in therapy or may require alteration of a few key residues. Sequence differences between rodent antibodies and human sequences can be minimized by replacing residues which differ from those in the human sequences by site directed mutagenesis of individual residues or by grating of entire complementarity determining regions. Alternatively, one can produce humanized antibodies using recombinant methods, as described in GB2188638B. Antibodies which specifically bind to a human lysostaphin-like

protease polypeptide can contain antigen binding sites which are either partially or fully humanized, as disclosed in U.S. Patent 5,565,332.

Alternatively, techniques described for the production of single chain antibodies can be adapted using methods known in the art to produce single chain antibodies which specifically bind to human lysostaphin-like protease polypeptides. Antibodies with related specificity, but of distinct idiotypic composition, can be generated by chain shuffling from random combinatorial immunoglobin libraries (Burton, *Proc. Natl. Acad. Sci. 88*, 11120-23, 1991).

Single-chain antibodies also can be constructed using a DNA amplification method, such as PCR, using hybridoma cDNA as a template (Thirion *et al.*, 1996, *Eur. J. Cancer Prev. 5*, 507-11). Single-chain antibodies can be mono- or bispecific, and can be bivalent or tetravalent. Construction of tetravalent, bispecific single-chain antibodies is taught, for example, in Coloma & Morrison, 1997, *Nat. Biotechnol. 15*, 159-63. Construction of bivalent, bispecific single-chain antibodies is taught in Mallender & Voss, 1994, *J. Biol. Chem. 269*, 199-206.

A nucleotide sequence encoding a single-chain antibody can be constructed using manual or automated nucleotide synthesis, cloned into an expression construct using standard recombinant DNA methods, and introduced into a cell to express the coding sequence, as described below. Alternatively, single-chain antibodies can be produced directly using, for example, filamentous phage technology. Verhaar *et al.*, 1995, *Int. J. Cancer 61*, 497-501; Nicholls *et al.*, 1993, *J. Immunol. Meth. 165*, 81-91.

Antibodies which specifically bind to human lysostaphin-like protease polypeptides also can be produced by inducing *in vivo* production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature (Orlandi *et al.*, *Proc. Natl. Acad. Sci. 86*, 3833-37, 1989; Winter *et al.*, *Nature 349*, 293-99, 1991).

Other types of antibodies can be constructed and used therapeutically in methods of the invention. For example, chimeric antibodies can be constructed as disclosed in WO 93/03151. Binding proteins which are derived from immunoglobulins and which are multivalent and multispecific, such as the "diabodies" described in WO 94/13804, also can be prepared.

Antibodies of the invention can be purified by methods well known in the art. For example, antibodies can be affinity purified by passage over a column to which a human lysostaphin-like protease polypeptide is bound. The bound antibodies can then be eluted from the column using a buffer with a high salt concentration.

Antisense Oligonucleotides

Antisense oligonucleotides are nucleotide sequences which are complementary to a specific DNA or RNA sequence. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form complexes and block either transcription or translation. Preferably, an antisense oligonucleotide is at least 11 nucleotides in length, but can be at least 12, 15, 20, 25, 30, 35, 40, 45, or 50 or more nucleotides long. Longer sequences also can be used. Antisense oligonucleotide molecules can be provided in a DNA construct and introduced into a cell as described above to decrease the level of human lysostaphin-like protease gene products in the cell.

Antisense oligonucleotides can be deoxyribonucleotides, ribonucleotides, or a combination of both. Oligonucleotides can be synthesized manually or by an automated synthesizer, by covalently linking the 5' end of one nucleotide with the 3' end of another nucleotide with non-phosphodiester internucleotide linkages such alkylphosphonates, phosphorothioates, phosphorodithioates, alkylphosphonothioates, alkylphosphonates, phosphoramidates, phosphate esters, carbamates, acetamidate, carboxymethyl esters, carbonates, and phosphate triesters. *See* Brown, *Meth. Mol.*

Biol. 20, 1-8, 1994; Sonveaux, *Meth. Mol. Biol. 26*, 1-72, 1994; Uhlmann *et al.*, *Chem. Rev. 90*, 543-83, 1990.

Modifications of human lysostaphin-like protease gene expression can be obtained by designing antisense oligonucleotides which will form duplexes to the control, 5', or regulatory regions of the human lysostaphin-like protease gene. Oligonucleotides derived from the transcription initiation site, *e.g.*, between positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or chaperons. Therapeutic advances using triplex DNA have been described in the literature (*e.g.*, Gee *et al.*, in Huber & Carr, MOLECULAR AND IMMUNOLOGIC APPROACHES, Futura Publishing Co., Mt. Kisco, N.Y., 1994). An antisense oligonucleotide also can be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Precise complementarity is not required for successful duplex formation between an antisense oligonucleotide and the complementary sequence of a human lysostaphin-like protease polynucleotide. Antisense oligonucleotides which comprise, for example, 2, 3, 4, or 5 or more stretches of contiguous nucleotides which are precisely complementary to a human lysostaphin-like protease polynucleotide, each separated by a stretch of contiguous nucleotides which are not complementary to adjacent human lysostaphin-like protease nucleotides, can provide targeting specificity for human lysostaphin-like protease mRNA. Preferably, each stretch of complementary contiguous nucleotides is at least 4, 5, 6, 7, or 8 or more nucleotides in length. Non-complementary intervening sequences are preferably 1, 2, 3, or 4 nucleotides in length. One skilled in the art can easily use the calculated melting point of an antisense-sense pair to determine the degree of mismatching which will be tolerated between a particular antisense oligonucleotide and a particular human lysostaphin-like protease polynucleotide sequence.

Antisense oligonucleotides can be modified without affecting their ability to hybridize to a human lysostaphin-like protease polynucleotide. These modifications can be internal or at one or both ends of the antisense molecule. For example, internucleoside phosphate linkages can be modified by adding cholesteryl or diamine moieties with varying numbers of carbon residues between the amino groups and terminal ribose. Modified bases and/or sugars, such as arabinose instead of ribose, or a 3', 5'-substituted oligonucleotide in which the 3' hydroxyl group or the 5' phosphate group are substituted, also can be employed in a modified antisense oligonucleotide. These modified oligonucleotides can be prepared by methods well known in the art. *See, e.g.*, Agrawal *et al.*, *Trends Biotechnol. 10*, 152-58, 1992; Uhlmann *et al.*, *Chem. Rev. 90*, 543-84, 1990; Uhlmann *et al.*, *Tetrahedron. Lett. 215*, 3539-42, 1987.

Ribozymes

Ribozymes are RNA molecules with catalytic activity. *See, e.g.*, Cech, *Science 236*, 1532-39; 1987; Cech, *Ann. Rev. Biochem. 59*, 543-68; 1990, Cech, *Curr. Opin. Struct. Biol. 2*, 605-09; 1992, Couture & Stinchcomb, *Trends Genet. 12*, 510-15, 1996. Ribozymes can be used to inhibit gene function by cleaving an RNA sequence, as is known in the art (*e.g.*, Haseloff *et al.*, U.S. Patent 5,641,673). The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of specific nucleotide sequences.

The coding sequence of a human lysostaphin-like protease polynucleotide can be used to generate ribozymes which will specifically bind to mRNA transcribed from the human lysostaphin-like protease polynucleotide. Methods of designing and constructing ribozymes which can cleave other RNA molecules in trans in a highly sequence specific manner have been developed and described in the art (*see* Haseloff

et al. Nature 334, 585-91, 1988). For example, the cleavage activity of ribozymes can be targeted to specific RNAs by engineering a discrete "hybridization" region into the ribozyme. The hybridization region contains a sequence complementary to the target RNA and thus specifically hybridizes with the target (see, for example, Gerlach *et al.*, EP 321,201).

Specific ribozyme cleavage sites within a human lysostaphin-like protease RNA target are initially identified by scanning the RNA molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the human lysostaphin-like protease target RNA containing the cleavage site can be evaluated for secondary structural features which may render the target inoperable. The suitability of candidate targets also can be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays. Longer complementary sequences can be used to increase the affinity of the hybridization sequence for the target. The hybridizing and cleavage regions of the ribozyme can be integrally related; thus, upon hybridizing to the human lysostaphin-like protease target RNA through the complementary regions, the catalytic region of the ribozyme can cleave the target.

Ribozymes can be introduced into cells as part of a DNA construct. Mechanical methods, such as microinjection, liposome-mediated transfection, electroporation, or calcium phosphate precipitation, can be used to introduce a ribozyme-containing DNA construct into cells in which it is desired to decrease human lysostaphin-like protease expression. Alternatively, if it is desired that the cells stably retain the DNA construct, it can be supplied on a plasmid and maintained as a separate element or integrated into the genome of the cells, as is known in the art. The DNA construct can include transcriptional regulatory elements, such as a promoter element, an enhancer or UAS element, and a transcriptional terminator signal, for controlling transcription of ribozymes in the cells.

As taught in Haseloff *et al.*, U.S. Patent 5,641,673, ribozymes can be engineered so that ribozyme expression will occur in response to factors which induce expression of a target gene. Ribozymes also can be engineered to provide an additional level of regulation, so that destruction of human lysostaphin-like protease mRNA occurs only when both a ribozyme and a target gene are induced in the cells.

Screening Methods

The invention provides methods for identifying modulators, *i.e.*, candidate or test compounds which bind to human lysostaphin-like protease polypeptides or polynucleotides and/or have a stimulatory or inhibitory effect on, for example, expression or activity of the human lysostaphin-like protease polypeptide or polynucleotide. Such compounds can be used, *e.g.*, to regulate degradation of extracellular matrix proteins such as elastin. Decreased extracellular matrix degradation is useful for preventing or suppressing malignant cells from metastasizing. Increased extracellular matrix degradation may be desired, for example, in developmental disorders characterized by inappropriately low levels of extracellular matrix degradation or in regeneration.

The invention provides assays for screening test compounds which bind to or modulate the activity of a human lysostaphin-like protease polypeptide or a human lysostaphin-like protease polynucleotide. A test compound preferably binds to a human lysostaphin-like protease polypeptide or polynucleotide. More preferably, a test compound decreases a human lysostaphin-like protease activity of a human lysostaphin-like protease polypeptide or expression of a human lysostaphin-like protease polynucleotide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the test compound.

Test Compounds

Test compounds can be pharmacologic agents already known in the art or can be compounds previously unknown to have any pharmacological activity. The compounds can be naturally occurring or designed in the laboratory. They can be isolated from microorganisms, animals, or plants, and can be produced recombinantly, or synthesized by chemical methods known in the art. If desired, test compounds can be obtained using any of the numerous combinatorial library methods known in the art, including but not limited to, biological libraries, spatially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead one-compound" library method, and synthetic library methods using affinity chromatography selection. The biological library approach is limited to polypeptide libraries, while the other four approaches are applicable to polypeptide, non-peptide oligomer, or small molecule libraries of compounds. *See* Lam, *Anticancer Drug Des. 12*, 145, 1997.

Methods for the synthesis of molecular libraries are well known in the art (*see*, for example, DeWitt *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 90*, 6909, 1993; Erb *et al. Proc. Natl. Acad. Sci. U.S.A. 91*, 11422, 1994; Zuckermann *et al.*, *J. Med. Chem. 37*, 2678, 1994; Cho *et al.*, *Science 261*, 1303, 1993; Carell *et al.*, *Angew. Chem. Int. Ed. Engl. 33*, 2059, 1994; Carell *et al.*, *Angew. Chem. Int. Ed. Engl. 33*, 2061; Gallop *et al.*, *J. Med. Chem. 37*, 1233, 1994). Libraries of compounds can be presented in solution (*see, e.g.*, Houghten, *BioTechniques 13*, 412-21, 1992), or on beads (Lam, *Nature 354*, 82-84, 1991), chips (Fodor, *Nature 364*, 555-56, 1993), bacteria or spores (Ladner, U.S. Patent 5,223,409), plasmids (Cull *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 89*, 1865-69, 1992), or phage (Scott & Smith, *Science 249*, 386-90, 1990; Devlin, *Science 249*, 404-06, 1990); Cwirla *et al.*, *Proc. Natl. Acad. Sci. 97*, 6378-82, 1990; Felici, *J. Mol. Biol. 222*, 301-10, 1991; and Ladner, U.S. Patent 5,223,409).

High Throughput Screening

Test compounds can be screened for the ability to bind to human lysostaphin-like protease polypeptides or polynucleotides or to affect human lysostaphin-like protease activity or human lysostaphin-like protease gene expression using high throughput screening. Using high throughput screening, many discrete compounds can be tested in parallel so that large numbers of test compounds can be quickly screened. The most widely established techniques utilize 96-well microtiter plates. The wells of the microtiter plates typically require assay volumes that range from 50 to 500 µl. In addition to the plates, many instruments, materials, pipettors, robotics, plate washers, and plate readers are commercially available to fit the 96-well format.

Alternatively, "free format assays," or assays that have no physical barrier between samples, can be used. For example, an assay using pigment cells (melanocytes) in a simple homogeneous assay for combinatorial peptide libraries is described by Jayawickreme *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 19,* 1614-18 (1994). The cells are placed under agarose in petri dishes, then beads that carry combinatorial compounds are placed on the surface of the agarose. The combinatorial compounds are partially released the compounds from the beads. Active compounds can be visualized as dark pigment areas because, as the compounds diffuse locally into the gel matrix, the active compounds cause the cells to change colors.

Another example of a free format assay is described by Chelsky, "Strategies for Screening Combinatorial Libraries: Novel and Traditional Approaches," reported at the First Annual Conference of The Society for Biomolecular Screening in Philadelphia, Pa. (Nov. 7-10, 1995). Chelsky placed a simple homogenous enzyme assay for carbonic anhydrase inside an agarose gel such that the enzyme in the gel would cause a color change throughout the gel. Thereafter, beads carrying combinatorial compounds via a photolinker were placed inside the gel and the compounds were partially released by UV-light. Compounds that inhibited the enzyme were observed as local zones of inhibition having less color change.

Yet another example is described by Salmon *et al.*, *Molecular Diversity 2*, 57-63 (1996). In this example, combinatorial libraries were screened for compounds that had cytotoxic effects on cancer cells growing in agar.

Another high throughput screening method is described in Beutel *et al.*, U.S. Patent 5,976,813. In this method, test samples are placed in a porous matrix. One or more assay components are then placed within, on top of, or at the bottom of a matrix such as a gel, a plastic sheet, a filter, or other form of easily manipulated solid support. When samples are introduced to the porous matrix they diffuse sufficiently slowly, such that the assays can be performed without the test samples running together.

Binding Assays

For binding assays, the test compound is preferably a small molecule which binds to and occupies the metalloprotease domain of the human lysostaphin-like protease polypeptide, thereby making the domain inaccessible to substrate such that normal biological activity is prevented. Examples of such small molecules include, but are not limited to, small peptides or peptide-like molecules. In binding assays, either the test compound or the human lysostaphin-like protease polypeptide can comprise a detectable label, such as a fluorescent, radioisotopic, chemiluminescent, or enzymatic label, such as horseradish peroxidase, alkaline phosphatase, or luciferase. Detection of a test compound which is bound to the human lysostaphin-like protease polypeptide can then be accomplished, for example, by direct counting of radio-emmission, by scintillation counting, or by determining conversion of an appropriate substrate to a detectable product.

Alternatively, binding of a test compound to a human lysostaphin-like protease polypeptide can be determined without labeling either of the interactants. For example, a microphysiometer can be used to detect binding of a test compound with a target polypeptide. A microphysiometer (*e.g.*, Cytosensor™) is an analytical

instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a test compound and a human lysostaphin-like protease polypeptide. (McConnell *et al.*, *Science 257*, 1906-12, 1992).

Determining the ability of a test compound to bind to a human lysostaphin-like protease polypeptide also can be accomplished using a technology such as real-time Bimolecular Interaction Analysis (BIA). Sjolander & Urbaniczky, *Anal. Chem. 63*, 2338-45, 1991, and Szabo *et al.*, *Curr. Opin. Struct. Biol. 5*, 699-705, 1995. BIA is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore™). Changes in the optical phenomenon surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In yet another aspect of the invention, a human lysostaphin-like protease polypeptide can be used as a "bait protein" in a two-hybrid assay or three-hybrid assay (see, *e.g.*, U.S. Patent 5,283,317; Zervos *et al.*, *Cell 72*, 223-32, 1993; Madura *et al.*, *J. Biol. Chem. 268*, 12046-54, 1993; Bartel *et al.*, *BioTechniques 14*, 920-24, 1993; Iwabuchi *et al.*, *Oncogene 8*, 1693-96, 1993; and Brent WO 94/10300), to identify other proteins which bind to or interact with the human lysostaphin-like protease polypeptide and modulate its activity.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. For example, in one construct a polynucleotide encoding a human lysostaphin-like protease polypeptide is fused to a polynucleotide encoding the DNA binding domain of a known transcription factor (*e.g.*, GAL-4). In the other construct, a DNA sequence that encodes an unidentified protein ("prey" or "sample") is fused to a polynucleotide that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are

able to interact *in vivo* to form an protein-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (*e.g.*, LacZ), which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected, and cell colonies containing the functional transcription factor can be isolated and used to obtain the DNA sequence encoding the protein which interacts with the human lysostaphin-like protease polypeptide.

It may be desirable to immobilize either the human lysostaphin-like protease polypeptide (or polynucleotide) or the test compound to facilitate separation of bound from unbound forms of one or both of the interactants, as well as to accommodate automation of the assay. Thus, either the human lysostaphin-like protease polypeptide (or polynucleotide) or the test compound can be bound to a solid support. Suitable solid supports include, but are not limited to, glass or plastic slides, tissue culture plates, microtiter wells, tubes, silicon chips, or particles such as beads (including, but not limited to, latex, polystyrene, or glass beads). Any method known in the art can be used to attach the human lysostaphin-like protease polypeptide (or polynucleotide) or test compound to a solid support, including use of covalent and non-covalent linkages, passive absorption, or pairs of binding moieties attached respectively to the polypeptide or test compound and the solid support. Test compounds are preferably bound to the solid support in an array, so that the location of individual test compounds can be tracked. Binding of a test compound to a human lysostaphin-like protease polypeptide (or polynucleotide) can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and microcentrifuge tubes.

In one embodiment, a human lysostaphin-like protease polypeptide is a fusion protein comprising a domain that allows the human lysostaphin-like protease polypeptide to be bound to a solid support. For example, glutathione-S-transferase fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical,

St. Louis, Mo.) or glutathione derivatized microtiter plates, which are then combined with the test compound or the test compound and the non-adsorbed human lysostaphin-like protease polypeptide; the mixture is then incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components. Binding of the interactants can be determined either directly or indirectly, as described above. Alternatively, the complexes can be dissociated from the solid support before binding is determined.

Other techniques for immobilizing polypeptides or polynucleotides on a solid support also can be used in screening assays of the invention. For example, either a human lysostaphin-like protease polypeptide (or polynucleotide) or a test compound can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated human lysostaphin-like protease polypeptides or test compounds can be prepared from biotin-NHS(N-hydroxysuccinimide) using techniques well known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.) and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies which specifically bind to a human lysostaphin-like protease polypeptide polynucleotides, or a test compound, but which do not interfere with a desired binding site, such as the metalloprotease domain of the human lysostaphin-like protease polypeptide, can be derivatized to the wells of the plate. Unbound target or protein can be trapped in the wells by antibody conjugation.

Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies which specifically bind to the human lysostaphin-like protease polypeptide (or polynucleotides) or test compound, enzyme-linked assays which rely on detecting a human lysostaphin-like protease activity of the human lysostaphin-like protease polypeptide, and SDS gel electrophoresis under non-reducing conditions.

Screening for test compounds which bind to a human lysostaphin-like protease polypeptide or polynucleotide also can be carried out in an intact cell. Any cell which comprises a human lysostaphin-like protease polynucleotide or polypeptide can be used in a cell-based assay system. A human lysostaphin-like protease polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Either a primary culture or an established cell line, including neoplastic cell lines such as the colon cancer cell lines HCT116, DLD1, HT29, Caco2, SW837, SW480, and RKO, breast cancer cell lines 21-PT, 21-MT, MDA-468, SK-BR3, and BT-474, the A549 lung cancer cell line, and the H392 glioblastoma cell line, or cells which have been transfected to express a human lysostaphin-like protease polypeptide can be used. An intact cell is contacted with a test compound. Binding of the test compound to a human lysostaphin-like protease polypeptide or polynucleotide is determined as described above, after lysing the cell to release the human lysostaphin-like protease polypeptide-test compound complex.

Enzyme Assays

Test compounds can be tested for the ability to increase or decrease a human lysostaphin-like protease activity of a human lysostaphin-like protease polypeptide. The ability of human lysostaphin-like protease to degrade elastin can be measured, for example, using the method described in Example 2. Human lysostaphin-like protease activity can be measured after contacting either a purified human lysostaphin-like protease polypeptide, a cell extract, or an intact cell with a test compound. A test compound which decreases human lysostaphin-like protease activity by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential agent for decreasing extracellular matrix degradation. A test compound which increases human lysostaphin-like protease activity by at least about 10, preferably about 50, more preferably about 75, 90, or 100% is identified as a potential agent for increasing extracellular matrix degradation.

Gene Expression

In another embodiment, test compounds which increase or decrease human lysostaphin-like protease gene expression are identified. A human lysostaphin-like protease polynucleotide is contacted with a test compound, and the expression of an RNA or polypeptide product of the human lysostaphin-like protease polynucleotide is determined. The level of expression of human lysostaphin-like protease mRNA or polypeptide in the presence of the test compound is compared to the level of expression of human lysostaphin-like protease mRNA or polypeptide in the absence of the test compound. The test compound can then be identified as a modulator of expression based on this comparison. For example, when expression of human lysostaphin-like protease mRNA or polypeptide is greater in the presence of the test compound than in its absence, the test compound is identified as a stimulator or enhancer of human lysostaphin-like protease mRNA or polypeptide is less expression. Alternatively, when expression of the mRNA or protein is less in the presence of the test compound than in its absence, the test compound is identified as an inhibitor of human lysostaphin-like protease mRNA or polypeptide expression.

The level of human lysostaphin-like protease mRNA or polypeptide expression in the cells can be determined by methods well known in the art for detecting mRNA or protein. Either qualitative or quantitative methods can be used. The presence of polypeptide products of a human lysostaphin-like protease polynucleotide can be determined, for example, using a variety of techniques known in the art, including immunochemical methods such as radioimmunoassay, Western blotting, and immunohistochemistry. Alternatively, polypeptide synthesis can be determined *in vivo*, in a cell culture, or in an *in vitro* translation system by detecting incorporation of labeled amino acids into a human lysostaphin-like protease polypeptide.

Such screening can be carried out either in a cell-free assay system or in an intact cell. Any cell which expresses a human lysostaphin-like protease polynucleotide can be used in a cell-based assay system. The human lysostaphin-like protease

polynucleotide can be naturally occurring in the cell or can be introduced using techniques such as those described above. Either a primary culture or an established cell line, including neoplastic cell lines such as the colon cancer cell lines HCT116, DLD1, HT29, Caco2, SW837, SW480, and RKO, breast cancer cell lines 21-PT, 21-MT, MDA-468, SK-BR3, and BT-474, the A549 lung cancer cell line, and the H392 glioblastoma cell line, as well as cells transferred with a human lysostaphin-like protease expression construct can be used.

Pharmaceutical Compositions

The invention also provides pharmaceutical compositions which can be administered to a patient to achieve a therapeutic effect. Pharmaceutical compositions of the invention can comprise a human lysostaphin-like protease polypeptide, human lysostaphin-like protease polynucleotide, antibodies which specifically bind to a human lysostaphin-like protease polypeptide, or mimetics, agonists, antagonists, or inhibitors of a human lysostaphin-like protease polypeptide. The compositions can be administered alone or in combination with at least one other agent, such as stabilizing compound, which can be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions can be administered to a patient alone, or in combination with other agents, drugs or hormones.

In addition to the active ingredients, these pharmaceutical compositions can contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Pharmaceutical compositions of the invention can be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, parenteral, topical, sublingual, or rectal means. Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for

oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combination of active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents can be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid, or a salt thereof, such as sodium alginate.

Dragee cores can be used in conjunction with suitable coatings, such as concentrated sugar solutions, which also can contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments can be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, *i.e.*, dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with a filler or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds can be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration can be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions can contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds can be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Non-lipid polycationic amino polymers also can be used for delivery. Optionally, the suspension also can contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention can be manufactured in a manner that is known in the art, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. The pharmaceutical composition can be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation can be a lyophilized powder which can contain any or all of the following: 1-50 mM histidine, 0.1%-2% sucrose, and 2-7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

Further details on techniques for formulation and administration can be found in the latest edition of REMINGTON'S PHARMACEUTICAL SCIENCES (Maack Publishing Co., Easton, Pa.). After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated

condition. Such labeling would include amount, frequency, and method of administration.

Therapeutic Indications and Methods

1. Tumor Cell Invasion and Metastasis. Cancer is a disease fundamentally caused by oncogenic cellular transformation. There are several hallmarks of transformed cells that distinguish them from their normal counterparts and underlie the pathophysiology of cancer. These include uncontrolled cellular proliferation, unresponsiveness to normal death-inducing signals (immortalization), increased cellular motility and invasiveness, increased ability to recruit blood supply through induction of new blood vessel formation (angiogenesis), genetic instability, and dysregulated gene expression. Various combinations of these aberrant physiologies, along with the acquisition of drug-resistance frequently lead to an intractable disease state in which organ failure and patient death ultimately ensue.

Most standard cancer therapies target cellular proliferation and rely on the differential proliferative capacities between transformed and normal cells for their efficacy. This approach is hindered by the facts that several important normal cell types are also highly proliferative and that cancer cells frequently become resistant to these agents. Thus, the therapeutic indices for traditional anti-cancer therapies rarely exceed 2.0.

The advent of genomics-driven molecular target identification has opened up the possibility of identifying new cancer-specific targets for therapeutic intervention that will provide safer, more effective treatments for cancer patients. Thus, newly discovered tumor-associated genes and their products can be tested for their role(s) in disease and used as tools to discover and develop innovative therapies. Genes playing important roles in any of the physiological processes outlined above can be characterized as cancer targets.

Genes or gene fragments identified through genomics can readily be expressed in one or more heterologous expression systems to produce functional recombinant proteins. These proteins are characterized *in vitro* for their biochemical properties and then used as tools in high-throughput molecular screening programs to identify chemical modulators of their biochemical activities. Agonists and/or antagonists of target protein activity can be identified in this manner and subsequently tested in cellular and *in vivo* disease models for anti-cancer activity. Optimization of lead compounds with iterative testing in biological models and detailed pharmacokinetic and toxicological analyses form the basis for drug development and subsequent testing in humans.

The human lysostaphin-like protease gene provides a therapeutic target for decreasing extracellular matrix degradation, in particular for treating or preventing metastatic cancer (10, 11). Cancers whose metastasis can be suppressed according to the invention include adenocarcinoma, melanoma, cancers of the adrenal gland, bladder, bone, breast, cervix, gall bladder, liver, lung, ovary, pancreas, prostate, testis, and uterus. Elastin has been identified within the meninges and the microvasculature of the normal human brain. The role that elastin plays in either facilitating astrocytoma cell attachment to these structures or modulating astrocytoma invasion has been characterized. There are also reports that elastin receptor may be involved in processes which regulate regional astrocytoma invasion. Thus, by regulating elastin degradation, regulators of human lysostaphin-like protease can be used to treat astrocytomas.

Circulating tumor cells arrested in the capillary beds of different organs must invade the endothelial cell lining and degrade its underlying basement membrane (BM) in order to invade into the extravascular tissue(s) where they establish metastasis (1, 2). Metastatic tumor cells often attach at or near the

intercellular junctions between adjacent endothelial cells. Such attachment of the metastatic cells is followed by rupture of the junctions, retraction of the endothelial cell borders and migration through the breach in the endothelium toward the exposed underlying BM (1).

Once located between endothelial cells and the BM, the invading cells must degrade the subendothelial glycoproteins and proteoglycans of the BM in order to migrate out of the vascular compartment. Several cellular enzymes (*e.g.*, collagenase IV, plasminogen activator, cathepsin B, elastase) are thought to be involved in degradation of BM (2). Suppression of human lysostaphin-like protease activity therefore can be used to suppress tumor cell invasion and metastasis.

2. Tumor Angiogenesis. Basic fibroblast growth factor (bFGF) has been extracted from the subendothelial extracellular matrix produced *in vitro* (3) and from basement membranes of the cornea (4), suggesting that extracellular matrix may serve as a reservoir for bFGF. Immunohistochemical staining revealed the localization of bFGF in basement membranes of diverse tissues and blood vessels (5). Despite the ubiquitous presence of bFGF in normal tissues, endothelial cell proliferation in these tissues is usually very low, which suggests that bFGF is somehow sequestered from its site of action. It is possible, therefore, that suppression of human lysostaphin-like protease activity can suppress release of active bFGF from extracellular matrix and basement membranes. In addition, displacement of bFGF from its storage within basement membranes and extracellular matrix may therefore provide a novel mechanism for induction of neovascularization in normal and pathological situations. Restriction of endothelial cell growth factors in the extracellular matrix may prevent their systemic action on the vascular endothelium, thus maintaining a very low rate of endothelial cells turnover and vessel growth. On the other hand, release of bFGF from storage in the extracellular matrix may elicit localized endothelial cell proliferation and

neovascularization in processes such as wound healing, inflammation and tumor development (6, 7).

3. Cardiovascular disease. Abdominal aortic aneurysms are characterized by degradation of the extracellular matrix, with a reduction in the elastin concentration of the arterial media. These changes have been linked to increased levels of endogenous metalloproteinases within the aorta (12, 13). Human lysostaphin-like protease is a potential therapeutic target for pharmacological inhibitors aimed at treating cardiovascular diseases including hypertension and aneurysm.

4. Inflammation and Cellular Immunity. Human lysostaphin-like protease activity may be involved in the ability of activated cells of the immune system to leave the circulation and elicit both inflammatory and autoimmune responses. Thus, inflammation and cellular immunity may be regulated by regulating activity of human lysostaphin-like protease.

5. Viral infection. Removal of the cell surface components by human lysostaphin-like protease may influence the ability of viruses to attach to the cell surface. Regulation of human lysostaphin-like protease may therefore be used to treat viral infections.

6. Neurodegenerative diseases. It is also possible that human lysostaphin-like protease activity can be used to degrade, for example, prion protein amyloid plaques of Genstmann-Straussler Syndrome, Creutzfeldt-Jakob disease, and Scrapie.

7. Restenosis and Atherosclerosis. Proliferation of arterial smooth muscle cells (SMCs) in response to endothelial injury and accumulation of cholesterol rich lipoproteins are basic events in the pathogenesis of atherosclerosis and restenosis (8). It is possible that human lysostaphin-like protease may be

involved in the catabolic pathway that may allow substantial cellular and interstitial accumulation of cholesterol rich lipoproteins. The latter pathway is expected to be highly atherogenic by promoting accumulation of apoB and apoE rich lipoproteins (*i.e.* LDL, VLDL, chylomicrons), independent of feedback inhibition by the cellular sterol content. Altered levels of human lysostaphin-like protease activity therefore may inhibit both SMC proliferation and lipid accumulation and thus may halt the progression of restenosis and atherosclerosis.

8. Other therapeutic and diagnostic indications. Anti-human lysostaphin-like protease antibodies can be applied for immunodetection and diagnosis of micrometastases, autoimmune lesions, and renal failure in biopsy specimens, plasma samples, and body fluids.

The invention further pertains to the use of novel agents identified by the screening assays described above. Accordingly, it is within the scope of this invention to use a test compound identified as described herein in an appropriate animal model. For example, an agent identified as described herein (*e.g.*, a modulating agent, an antisense nucleic acid molecule, a specific antibody, ribozyme, or a polypeptide-binding partner) can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

A reagent which affects human lysostaphin-like protease activity can be administered to a human cell, either *in vitro* or *in vivo*, to reduce human lysostaphin-like protease activity. The reagent preferably binds to an expression product of a human lysostaphin-like protease gene. If the expression product is a polypeptide, the reagent is preferably an antibody. For treatment of human cells *ex vivo*, an antibody can be added to a preparation of stem cells which have been removed from the body. The

cells can then be replaced in the same or another human body, with or without clonal propagation, as is known in the art.

In one embodiment, the reagent is delivered using a liposome. Preferably, the liposome is stable in the animal into which it has been administered for at least about 30 minutes, more preferably for at least about 1 hour, and even more preferably for at least about 24 hours. A liposome comprises a lipid composition that is capable of targeting a reagent, particularly a polynucleotide, to a particular site in an animal, such as a human. Preferably, the lipid composition of the liposome is capable of targeting to a specific organ of an animal, such as the lung or liver.

A liposome useful in the present invention comprises a lipid composition that is capable of fusing with the plasma membrane of the targeted cell to deliver its contents to the cell. Preferably, the transfection efficiency of a liposome is about 0.5 μg of DNA per 16 nmole of liposome delivered to about 10^6 cells, more preferably about 1.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells, and even more preferably about 2.0 μg of DNA per 16 nmol of liposome delivered to about 10^6 cells. Preferably, a liposome is between about 100 and 500 nm, more preferably between about 150 and 450 nm, and even more preferably between about 200 and 400 nm in diameter.

Suitable liposomes for use in the present invention include those liposomes standardly used in, for example, gene delivery methods known to those of skill in the art. More preferred liposomes include liposomes having a polycationic lipid composition and/or liposomes having a cholesterol backbone conjugated to polyethylene glycol. Optionally, a liposome comprises a compound capable of targeting the liposome to a tumor cell, such as a tumor cell ligand exposed on the outer surface of the liposome.

Complexing a liposome with a reagent such as an antisense oligonucleotide or ribozyme can be achieved using methods which are standard in the art (see, for

example, U.S. Patent 5,705,151). Preferably, from about 0.1 μg to about 10 μg of polynucleotide is combined with about 8 nmol of liposomes, more preferably from about 0.5 μg to about 5 μg of polynucleotides are combined with about 8 nmol liposomes, and even more preferably about 1.0 μg of polynucleotides is combined with about 8 nmol liposomes.

In another embodiment, antibodies can be delivered to specific tissues *in vivo* using receptor-mediated targeted delivery. Receptor-mediated DNA delivery techniques are taught in, for example, Findeis *et al. Trends in Biotechnol. 11*, 202-05 (1993); Chiou *et al.*, GENE THERAPEUTICS: METHODS AND APPLICATIONS OF DIRECT GENE TRANSFER (J.A. Wolff, ed.) (1994); Wu & Wu, *J. Biol. Chem. 263*, 621-24 (1988); Wu *et al.*, *J. Biol. Chem. 269*, 542-46 (1994); Zenke *et al.*, *Proc. Natl. Acad. Sci. U.S.A. 87*, 3655-59 (1990); Wu *et al.*, *J. Biol. Chem. 266*, 338-42 (1991).

If the reagent is a single-chain antibody, polynucleotides encoding the antibody can be constructed and introduced into a cell either *ex vivo* or *in vivo* using well-established techniques including, but not limited to, transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads, protoplast fusion, viral infection, electroporation, "gene gun," and DEAE- or calcium phosphate-mediated transfection.

Determination of a Therapeutically Effective Dose

The determination of a therapeutically effective dose is well within the capability of those skilled in the art. A therapeutically effective dose refers to that amount of active ingredient which increases or decreases extracellular matrix degradation relative to that which occurs in the absence of the therapeutically effective dose.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rabbits, dogs, or pigs. The

animal model also can be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

Therapeutic efficacy and toxicity, *e.g.*, ED_{50} (the dose therapeutically effective in 54% of the population) and LD_{50} (the dose lethal to 54% of the population), can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD_{50}/ED_{50}.

Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active ingredient or to maintain the desired effect. Factors which can be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions can be administered every 3 to 4 days, every week, or once every two weeks depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts can vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations

for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

Effective *in vivo* dosages of an antibody are in the range of about 5 µg to about 50 µg/kg, about 50 µg to about 5 mg/kg, about 100 µg to about 500 µg/kg of patient body weight, and about 200 to about 250 µg/kg of patient body weight. For administration of polynucleotides encoding single-chain antibodies, effective *in vivo* dosages are in the range of about 100 ng to about 200 ng, 500 ng to about 50 mg, about 1 µg to about 2 mg, about 5 µg to about 500 µg, and about 20 µg to about 100 µg of DNA.

If the expression product is mRNA, the reagent is preferably an antisense oligonucleotide or a ribozyme. Polynucleotides which express antisense oligonucleotides or ribozymes can be introduced into cells by a variety of methods, as described above.

Preferably, a reagent reduces expression of a human lysostaphin-like protease polynucleotide or activity of a human lysostaphin-like protease polypeptide by at least about 10, preferably about 50, more preferably about 75, 90, or 100% relative to the absence of the reagent. The effectiveness of the mechanism chosen to decrease the level of expression of a human lysostaphin-like protease polynucleotide or the activity of a human lysostaphin-like protease polypeptide can be assessed using methods well known in the art, such as hybridization of nucleotide probes to human lysostaphin-like protease-specific mRNA, quantitative RT-PCR, immunologic detection of a human lysostaphin-like protease polypeptide, or measurement of human lysostaphin-like protease activity.

In any of the embodiments described above, any of the pharmaceutical compositions of the invention can be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination

therapy can be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents can act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

Any of the therapeutic methods described above can be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

The above disclosure generally describes the present invention, and all patents and patent applications cited in this disclosure are expressly incorporated herein. A more complete understanding can be obtained by reference to the following specific examples which are provided for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLE 1

Identification of a test compound which binds to a human lysostaphin-like protease polypeptide

Purified human lysostaphin-like protease polypeptides comprising a glutathione-S-transferase protein and absorbed onto glutathione-derivatized wells of 96-well microtiter plates are contacted with test compounds from a small molecule library at pH 7.0 in a physiological buffer solution. Human lysostaphin-like protease polypeptides comprise the amino acid sequence shown in SEQ ID NO. 2. The test compounds comprise a fluorescent tag. The samples are incubated for 5 minutes to one hour. Control samples are incubated in the absence of a test compound.

The buffer solution containing the test compounds is washed from the wells. Binding of a test compound to a human lysostaphin-like protease polypeptide is

detected by fluorescence measurements of the contents of the wells. A test compound which increases the fluorescence in a well by at least 15% relative to fluorescence of a well in which a test compound was not incubated is identified as a compound which binds to a human lysostaphin-like protease polypeptide.

EXAMPLE 2

Identification of a test compound which decreases human lysostaphin-like protease activity

Cellular extracts from human cancer cell lines are contacted with test compounds from a small molecule library and assayed for elastin degradation by human lysostaphin-like protease as described in Park *et al.*, *Int. J. Biochem. Cell Biol. 27*, 139-46, 1995. Control extracts, in the absence of a test compound, also are assayed.

Bovine ligamentum nuchae elastin is radiolabeled by reductive alkylation using ^{3}H-sodium borohydride to a specific activity of about 1900 cpm/μg (Banda *et al.*, "Elastinolytic enzymes," in METHODS FOR STUDYING MONONUCLEAR PHAGOCYTES, Adams *et al.*, eds., pp. 603-18, Academic Press, NY, 1981). Samples are placed in 200 μl of TBS containing 0.01% Brij 58 and incubated with 80 μg ^{3}H-elastin for 2, 4, 7, 10, 20, and 25 hours. After termination of the assay by centrifugation, solubilized tritiated peptides are measured in a 100 μl aliquot of the reaction mixture by scintillation counting. Assays are conducted in triplicate, and the results are expressed as mean cpm +/- standard deviation.

A test compound which decreases human lysostaphin-like protease activity of the extract relative to the control extract by at least 20% is identified as a human lysostaphin-like protease inhibitor.

EXAMPLE 3

Identification of a test compound which decreases human lysostaphin-like protease gene expression

A test compound is administered to a culture of the breast tumor cell line MDA-468 and incubated at 37°C for 10 to 45 minutes. A culture of the same type of cells incubated for the same time without the test compound provides a negative control.

RNA is isolated from the two cultures as described in Chirgwin *et al.*, *Biochem. 18*, 5294-99, 1979). Northern blots are prepared using 20 to 30 µg total RNA and hybridized with a ^{32}P-labeled human lysostaphin-like protease-specific probe at 65°C in Express-hyb (CLONTECH). The probe comprises at least 11 contiguous nucleotides selected from the complement of SEQ ID NO. 1. A test compound which decreases the human lysostaphin-like protease -specific signal relative to the signal obtained in the absence of the test compound is identified as an inhibitor of human lysostaphin-like protease gene expression.

EXAMPLE 4

Treatment of a breast tumor with a reagent which specifically binds to a human lysostaphin-like protease gene product

Synthesis of antisense human lysostaphin-like protease oligonucleotides comprising at least 11 contiguous nucleotides selected from SEQ ID NO. 1 is performed on a Pharmacia Gene Assembler series synthesizer using the phosphoramidite procedure (Uhlmann *et al.*, *Chem. Rev. 90*, 534-83, 1990). Following assembly and deprotection, oligonucleotides are ethanol-precipitated twice, dried, and suspended in phosphate-buffered saline (PBS) at the desired concentration. Purity of these oligonucleotides is tested by capillary gel electrophoreses and ion exchange HPLC.

Endotoxin levels in the oligonucleotide preparation are determined using the *Limulus* Amebocyte Assay (Bang, *Biol. Bull. (Woods Hole, Mass.) 105*, 361-62, 1953).

An aqueous composition containing the antisense oligonucleotides at a concentration of 0.1-100 μM is injected directly into a breast tumor with a needle. The needle is placed in the tumors and withdrawn while expressing the aqueous composition within the tumor.

The breast tumor is monitored over a period of days or weeks. Additional injections of the antisense oligonucleotides can be given during that time. Metastasis of the breast tumor is suppressed due to decreased human lysostaphin-like protease activity of the breast tumor cells.

REFERENCES

1. Nicolson (1988) Organ specificity of tumor metastasis: Role of preferential adhesion, invasion and growth of malignant cells at specific secondary sites. *Cancer Met. Rev. 7*, 143-188.

2. Liotta *et al.* (1983) Tumor invasion and the extracellular matrix. *Lab. Invest. 49*, 639-649.

3. Vlodavsky *et al.* (1987) Endothelial cell-derived basic fibroblast growth factor: Synthesis and deposition into subendothelial extracellular matrix. *Proc. Natl. Acad. Sci. USA 84*, 2292-2296.

4 Folkman *et al.* (1980) A heparin-binding angiogenic protein--basic fibroblast growth factor--is stored within basement membrane. *Am. J. Pathol. 130, 393400.*

5. Cardon-Cardo *et al.* (1990) Expression of basic fibroblast growth factor in normal human tissues. *Lab. Invest. 63*, 832-840.

6. Vlodavsky *et al.* (1991) Extracellular sequestration and release of fibroblast growth factor: a regulatory mechanism? *Trends Biochem. Sci. 16*, 268-271.

7. Vlodavsky *et al.* (1993) Extracellular matrix-bound growth factors, enzymes and plasma proteins. In BASEMENT MEMBRANES: CELLULAR AND MOLECULAR ASPECTS Rohrbach & Timpl, eds., pp 327-343. Academic Press Inc., Orlando, Fla.

8. Ross (1993) The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature (Lond.). 362*, 801-809.

9. Park *et al.* (1995). Binding and degradation of elastin by the staphylolytic enzyme lysostaphin. *Int. J. Biochem. Cell. Biol. 27*, 139-46

10. Goel A, Chauhan SS (1997). Role of proteases in tumor invasion and metastasis. *Indian J .Exp. Biol. 35*, 553-64

11. Tímár *et al.* (1995). Interaction of tumour cells with elastin and the metastatic phenotype. *Ciba Found. Symp. 192*, 321-35

12. Treharne *et al.* (1999). Marimastat inhibits elastin degradation and matrix metalloprotease 2 activity in a model of aneurysm disease. *Br. J. Surg. 86*, 1053-8

13. Partridge *et al.* (1999). Long-term alcohol consumption increases matrix metalloprotease-2 activity in rat aorta. *Life Sci. 65*, 1395-402

CLAIMS

1. An isolated polynucleotide encoding a Lysostaphin-like protease polypeptide and being selected from the group consisting of:

a) a polynucleotide encoding a Lysostaphin-like protease polypeptide comprising an amino acid sequence selected from the group consisting of:
amino acid sequences which are at least about 54% identical to the amino acid sequence shown in SEQ ID NO. 2;
the amino acid sequence shown in SEQ ID NO. 2.

b) a polynucleotide comprising the sequence of SEQ ID NO. 1;

c) a polynucleotide which hybridizes under stringent conditions to a polynucleotide specified in (a) and (b);

d) a polynucleotide the sequence of which deviates from the polynucleotide sequences specified in (a) to (c) due to the degeneration of the genetic code; and

e) a polynucleotide which represents a fragment, derivative or allelic variation of a polynucleotide sequence specified in (a) to (d).

2. An expression vector containing any polynucleotide of claim 1.

3. A host cell containing the expression vector of claim 2.

4. A substantially purified Lysostaphin-like protease polypeptide encoded by a polynucleotide of claim 1.

5. A method for producing a Lysostaphin-like protease polypeptide, wherein the method comprises the following steps:

a) culturing the host cell of claim 3 under conditions suitable for the expression of the Lysostaphin-like protease polypeptide; and

b) recovering the Lysostaphin-like protease polypeptide from the host cell culture.

6. A method for detection of a polynucleotide encoding a Lysostaphin-like protease polypetide in a biological sample comprising the following steps:

hybridizing any polynucleotide of claim 1 to a nucleic acid material of a biological sample, thereby forming a hybridization complex; and

detecting said hybridization complex.

7. The method of claim 6, wherein before hybridization, the nucleic acid material of the biological sample is amplified.

8. A method for the detection of a polynucleotide of claim 1 or a Lysostaphin-like protease polypeptide of claim 4 comprising the steps of:

contacting a biological sample with a reagent which specifically interacts with the polynucleotide or the Lysostaphin-like protease polypeptide and detecting the interaction.

9. A diagnostic kit for conducting the method of any one of claims 6 to 8.

10. A method of screening for agents which decrease the activity of a Lysostaphin-like protease, comprising the steps of:

contacting a test compound with any Lysostaphin-like protease polypeptide encoded by any polynucleotide of claim 1;

detecting binding of the test compound to the Lysostaphin-like protease polypeptide, wherein a test compound which binds to the polypeptide is identified as a potential therapeutic agent for decreasing the activity of a Lysostaphin-like protease.

11. A method of screening for agents which regulate the activity of a Lysostaphin-like protease, comprising the steps of:

contacting a test compound with a Lysostaphin-like protease polypeptide encoded by any polynucleotide of claim 1; and

detecting a Lysostaphin-like protease activity of the polypeptide, wherein a test compound which increases the Lysostaphin-like protease activity is identified as a potential therapeutic agent for increasing the activity of the Lysostaphin-like protease, and wherein a test compound which decreases the Lysostaphin-like protease activity of the polypeptide is identified as a potential therapeutic agent for decreasing the activity of the Lysostaphin-like protease.

12. A method of screening for agents which decrease the activity of a Lysostaphin-like protease, comprising the steps of:

contacting a test compound with any polynucleotide of claim 1 and

detecting binding of the test compound to the polynucleotide, wherein a test compound which binds to the polynucleotide is identified as a potential therapeutic agent for decreasing the activity of Lysostaphin-like protease.

13. A method of reducing the activity of Lysostaphin-like protease, comprising the steps of:

contacting a cell with a reagent which specifically binds to any polynucleotide of claim 1 or any Lysostaphin-like protease polypeptide of claim 4, whereby the activity of Lysostaphin-like protease is reduced.

14. A reagent that modulates the activity of a Lysostaphin-like protease polypeptide or a polynucleotide wherein said reagent is identified by the method of any of the claims 10 to 12.

15. A pharmaceutical composition, comprising:

the expression vector of claim 2 or the reagent of claim 14 and a pharmaceutically acceptable carrier.

16. Use of the pharmaceutical composition of claim 15 for modulating the activity of a Lysostaphin-like protease in a disease.

17. Use of claim 16 wherein the disease is metastasis of malignant cells, tumor angiogenesis, inflammation, cardiovascular disease, including hypertension, aneurysmal dilatation, and atherosclerosis, neurodegenerative diseases, or pathogenic infections.

18. A cDNA encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 2

19. The cDNA of claim 18 which comprises SEQ ID NO. 1.

20. The cDNA of claim 18 which consists of SEQ ID NO.1.

21. An expression vector comprising a polynucleotide which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 2.

22. The expression vector of claim 21 wherein the polynucleotide consists of SEQ ID NO. 1.

23. A host cell comprising an expression vector which encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 2.

24. The host cell of claim 23 wherein the polynucleotide consists of SEQ ID NO. 1.

25. A purified polypeptide comprising the amino acid sequence shown in SEQ ID NO. 2.

26. The purified polypeptide of claim 25 which consists of the amino acid sequence shown in SEQ ID NO. 2.

27. A fusion protein comprising a polypeptide having the amino acid sequence shown in SEQ ID NO. 2.

28. A method of producing a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 2, comprising the steps of:

culturing a host cell comprising an expression vector which encodes the polypeptide under conditions whereby the polypeptide is expressed; and isolating the polypeptide.

29. The method of claim 28 wherein the expression vector comprises SEQ ID NO. 1.

30. A method of detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 2, comprising the steps of:

hybridizing a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO.1 to nucleic acid material of a biological sample, thereby forming a hybridization complex; and detecting the hybridization complex.

31. The method of claim 30 further comprising the step of amplifying the nucleic acid material before the step of hybridizing.

32. A kit for detecting a coding sequence for a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 2, comprising:

a polynucleotide comprising 11 contiguous nucleotides of SEQ ID NO. 1; and instructions for the method of claim 30.

33. A method of detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 2, comprising the steps of:

contacting a biological sample with a reagent that specifically binds to the polypeptide to form a reagent-polypeptide complex; and

detecting the reagent-polypeptide complex.

34. The method of claim 33 wherein the reagent is an antibody.

35. A kit for detecting a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 2, comprising:

an antibody which specifically binds to the polypeptide; and instructions for the method of claim 33.

36. A method of screening for agents which can regulate the activity of a Lysostaphin-like protease protein, comprising the steps of:

contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of: (1) amino acid sequences which are at least about 54% identical to the amino acid sequence shown in SEQ ID NO. 2 and (2) the amino acid sequence shown in SEQ ID NO. 2; and

detecting binding of the test compound to the polypeptide, wherein a test compound which binds to the polypeptide is identified as a potential agent for regulating activity of the Lysostaphin-like protease protein.

37. The method of claim 36 wherein the step of contacting is in a cell.

38. The method of claim 36 wherein the cell is *in vitro*.

39. The method of claim 36 wherein the step of contacting is in a cell-free system.

40. The method of claim 36 wherein the polypeptide comprises a detectable label.

41. The method of claim 36 wherein the test compound comprises a detectable label.

42. The method of claim 36 wherein the test compound displaces a labeled ligand which is bound to the polypeptide.

43. The method of claim 36 wherein the polypeptide is bound to a solid support.

44. The method of claim 36 wherein the test compound is bound to a solid support.

45. A method of screening for agents which regulate an activity of a human human Lysostaphin-like protease protein, comprising the steps of:

contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of: (1) amino acid sequences which are at least about 54% identical to the amino acid sequence shown in SEQ ID NO. 2 and (2) the amino acid sequence shown in SEQ ID NO. 2; and

detecting an activity of the polypeptide, wherein a test compound which increases the activity of the polypeptide is identified as a potential agent for increasing the activity of the human Lysostaphin-like protease protein, and wherein a test compound which decreases the activity of the polypeptide is identified as a potential agent for decreasing the activity of the human Lysostaphin-like protease protein.

46. The method of claim 45 wherein the step of contacting is in a cell.

47. The method of claim 45 wherein the cell is *in vitro*.

48. The method of claim 45 wherein the step of contacting is in a cell-free system.

49. The method of claim 45 wherein the activity is cyclic AMP formation.

50. The method of claim 45 wherein the activity is mobilization of intracellular calcium.

51. The method of claim 45 wherein the activity is phosphoinositide metabolism.

52. A method of screening for agents which regulate an activity of a human Lysostaphin-like protease protein, comprising the steps of:

contacting a test compound with a product encoded by a polynucleotide which comprises the nucleotide sequence shown in SEQ ID NO. 1; and

detecting binding of the test compound to the product, wherein a test compound which binds to the product is identified as a potential agent for regulating the activity of the human Lysostaphin-like protease protein.

53. The method of claim 52 wherein the product is a polypeptide.

54. The method of claim 52 wherein the product is RNA.

55. A method of reducing activity of a human Lysostaphin-like protease protein, comprising the step of:

contacting a cell with a reagent which specifically binds to a product encoded by a polynucleotide comprising the nucleotide sequence shown in SEQ ID NO. 1, whereby the activity of a human Lysostaphin-like protease protein is reduced.

56. The method of claim 55 wherein the product is a polypeptide.

57. The method of claim 56 wherein the reagent is an antibody.

58. The method of claim 55 wherein the product is RNA.

59. The method of claim 58 wherein the reagent is an antisense oligonucleotide.

60. The method of claim 58 wherein the reagent is a ribozyme.

61. The method of claim 55 wherein the cell is *in vitro*.

62. The method of claim 55 wherein the cell is *in vivo*.

63. A pharmaceutical composition, comprising:

a reagent which specifically binds to a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 2; and

a pharmaceutically acceptable carrier.

64. The pharmaceutical composition of claim 63 wherein the reagent is an antibody.

65. A pharmaceutical composition, comprising:

a reagent which specifically binds to a product of a polynucleotide comprising the nucleotide sequence shown in SEQ ID NO. 1; and

a pharmaceutically acceptable carrier.

66. The pharmaceutical composition of claim 65 wherein the reagent is a ribozyme.

67. The pharmaceutical composition of claim 65 wherein the reagent is an antisense oligonucleotide.

68. The pharmaceutical composition of claim 65 wherein the reagent is an antibody.

69. A pharmaceutical composition, comprising:

an expression vector encoding a polypeptide comprising the amino acid sequence shown in SEQ ID NO. 2; and

a pharmaceutically acceptable carrier.

70. The pharmaceutical composition of claim 69 wherein the expression vector comprises SEQ ID NO. 1.

71. A method of treating Lysostaphin-like protease, comprising the step of:

administering to a patient in need thereof a therapeutically effective dose of a reagent that inhibits a function of a human Lysostaphin-like protease protein, whereby symptoms of the Lysostaphin-like protease are ameliorated.

72. The method of claim 71 wherein the reagent is identified by the method of claim 36.

73. The method of claim 71 wherein the reagent is identified by the method of claim 45.

74. The method of claim 71 wherein the reagent is identified by the method of claim 52.

75. A method of treating a Lysostaphin-like protease disorder, comprising the step of:

administering to a patient in need thereof a therapeutically effective dose of a reagent that inhibits a function of a human Lysostaphin-like protease protein, whereby symptoms of the Lysostaphin-like protease disorder are ameliorated.

76. The method of claim 75 wherein the reagent is identified by the method of claim 36.

77. The method of claim 75 wherein the reagent is identified by the method of claim 45.

78. The method of claim 75 wherein the reagent is identified by the method of claim 52.

79. The method of claim 75 wherein the Lysostaphin-like protease disorder is metastasis of malignant cells, tumor angiogenesis, inflammation, cardiovascular disease, including hypertension, aneurysmal dilatation, and atherosclerosis, neurodegenerative diseases, or pathogenic infections.

Fig. 1

GCGGCCGCCG CCAGCACGCC GGGATCGATC TCGCCGCGCC
TACTGGCACC CCGGTCTACG CCACCGCCGA CGGCATCGTC
AGCCGCGCCG ACTGGTATTC GAGCTACGGC CTCTACATCA
GCGTCGAGCA TGGCGCCTCG ATGCAGACCC GCTACGCCCA
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AAGGGCGACC TCATCGGCTA TGTCGGCTCG ACCGGCCGTT
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TCTTGCAGTC AATCCGATTC CGTATATGGT GGAGAGCGAA
GCACAGCTGG CCTACGCC

Fig. 2

GRRQHAGIDL AAPTGTPVYA TADGIVSRAD WYSSYGLYIS
VEHGASMQTR YAHLSRLAVA AGDNVKKGDL IGYVGSTGRS
TGPHLHYEVR VEGLAVNPIP YMVESEAQLA YA

Fig. 3

MQSQSRIKDLKRKPGQGANLYNLSLGGLIIIGGLVWSLPGSTTG
NLEIEQSAPAPVTPAAPPAPVKATAPVPVQSTPDSKPAAPRLPA
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Fig. 4

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H:   283  RMHNGTDIGAPMGTPVLAAYDGIVAAAQWSGGYGLMVTLRHLDGTQESRYAHLSEAFVES

          GDNVKKGDLIGYVGSTGRSTGPHLHYEVR     272
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Val Lys Ala Thr Ala Pro Val Pro Val Gln Ser Thr Pro Asp Ser Lys
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Gln Pro Ala Gln Ile Arg Gln Gly Leu Gln Lys Asn Ser Tyr Ile Asp
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```
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Gln Gln Ser Ser Pro Val Asn Pro Pro Lys Ala Ile Thr Pro Arg Arg
                165                 170                 175

Gln Ser Ile Asn Ala Arg Asn Ala Ala Pro Gly Asn Asn Ser Val Ser
            180                 185                 190

Pro Leu Asn Pro Leu Asn Ile Gln Pro Lys Ile Ser Ser Leu Ala Ile
        195                 200                 205

Gly Gln Pro Val Gly Ser Asn Gln Val Phe Ser Leu Asp Pro Met Ala
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Asn Arg Gly Ile Gln Ile Ala Leu Ala Pro Leu Pro Glu Tyr Ser Arg
225                 230                 235                 240

Ala Thr Gly Leu Tyr Ser Thr Gln Gly Gln Pro Asn Gln Gly Thr Asp
                245                 250                 255

Leu Met Phe Pro Val Ala Gly Val Asn Pro Ile Thr Ser Ala Phe Gly
            260                 265                 270

Trp Arg Ile His Pro Ile Ser Gly Gln Gly Arg Met His Asn Gly Thr
        275                 280                 285

Asp Ile Gly Ala Pro Met Gly Thr Pro Val Leu Ala Ala Tyr Asp Gly
    290                 295                 300

Ile Val Ala Ala Ala Gln Trp Ser Gly Gly Tyr Gly Leu Met Val Thr
305                 310                 315                 320

Leu Arg His Leu Asp Gly Thr Gln Glu Ser Arg Tyr Ala His Leu Ser
                325                 330                 335

Glu Ala Phe Val Glu Ser Gly Gln Gln Val Thr Gln Gly Glu Val Ile
            340                 345                 350

Gly Arg Val Gly Ser Thr Gly Phe Ser Thr Gly Pro His Leu His Phe
        355                 360                 365

Glu Trp Arg His Leu Thr Asn Asp Gly Trp Val Ala Val Asp Ala Gly
    370                 375                 380

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